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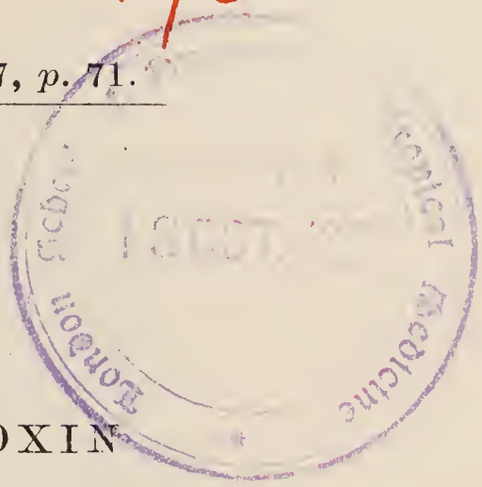

THE TITRATION OF SCARLET FEVER ANTITOXIN IN RABBITS.

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IT is generally admitted that one of the most urgent needs in connexion with recent work on scarlet fever is a method of titrating toxin and antitoxin by some means other than on human subjects. Up to now the only promise of an animal test has come from the researches on goats of Kirkbride and Wheeler,[1] but the work has not yet been generally confirmed and the method is complicated by many difficulties.

Many attempts have been made in the past to measure in various laboratory animals, particularly mice and rabbits, the protective value of antibacterial sera against living cultures of streptococci. The results of most of this work in our own and other workers' experience have been disappointing. It has been found, however, that rabbits could be killed with reasonable regularity by the intravenous injection of the "Dochez" streptococcus and certain other scarlet fever strains. If the rabbits were injected intravenously 4 to 6 hours previously with an adequate amount of scarlet fever antitoxin, death did not take place or was delayed beyond the sixth day. 10 c.cm. of a 20-hour tryptic digest broth culture of the *Streptococcus scarlatinæ*, given intravenously, killed in successive experiments 70 per cent. of 63 rabbits of medium weight within 48 hours and 92 per cent. within six days. 5 c.cm. of unconcentrated and 1 c.cm. or less of concentrated scarlet antitoxin protected the great majority of rabbits. The protected animals, which survived for a longer

[1] Kirkbride, Mary B., and Wheeler, Mary W.: Proc. Soc. Exper. Biol. and Med., 1924, xxii., 86.

period than six days, almost always developed a purulent arthritis of one or more joints, from which a pure culture of the streptococcus could usually be obtained.

With sera other than streptococcal no protection was obtained; with various antistreptococcal sera, other than scarlatinal, from 17 to 29 per cent. of the rabbits were protected by doses of 5 c.cm. of serum. This group included unconcentrated sera prepared against streptococci of erysipelas, puerperal fever, and pyogenic infections. Seventeen rabbits were injected with 5 c.cm. of a serum prepared by immunisation with filtrates of a strain of *Streptococcus pyogenes*. Of these, 29 per cent. survived. This strain was isolated by Sir Frederick Andrewes from the septic finger of a nurse at St. Bartholomew's Hospital. It produced a toxin which gave a reaction indistinguishable from the Dick reaction in Dick-positive individuals, but no reaction in Dick-negative individuals; it had, therefore, a close antigenic relationship to the scarlet fever group of streptococci.

Having found that specific scarlet fever antitoxins could be distinguished from other sera, an attempt was made to compare scarlet fever antitoxins of different values as tested by the Schultz-Charlton and passive immunity methods. Three pairs of sera which had been placed by means of human tests in "order of merit" were compared. Of two unconcentrated sera, "A" was considered better than "B" on human tests. Serum "A" in a dose of 10 c.cm. turned 13 out of 14 "Dick-positive reactors" negative within 24 hours, and 0·2 c.cm. of a 1 in 500 dilution blanched the rashes of 7 out of 15 patients. Serum "B" turned none of 4 patients Dick-negative, and blanched poorly the rashes of 4 out of 15 patients. In the rabbit test 15 rabbits were injected with each of the two sera in corresponding doses—viz., 10 c.cm., 5 c.cm., and 1 c.cm. Ten of the rabbits in the "A" batch survived, but 5 only in the "B" batch.

The second pair of sera consisted of a concentrated batch, "D," and an unconcentrated, "E." By human tests it was found that with 2·5 c.cm. of serum "D," ten Dick-positive reactors became negative over night, and still gave a negative Dick test when retested on the twelfth day, whereas serum "E" in a dose of 10 c.cm. turned only 7 out of 10 patients negative. In the rabbit test the percentages of survivals were at every dose higher for serum "D" than for serum "E." A dose of 1 c.cm. of serum "D" saved all three rabbits injected,

while it took 10 c.cm. of serum " E " to produce the same result. With a third pair of sera the same agreement between human and rabbit tests was found.

We have not yet had an opportunity of comparing sera known to be efficient in the treatment of scarlet fever with sera which proved unsatisfactory, though this is the crucial test of any method of titration.

In analysing these results, it was obviously most important to decide whether the protection of the rabbits was due to antitoxin per se, or to some other protective antibody produced in the horse in response to immunisation.

The following observations have a bearing on this question : (1) Many of the sera were experimentally made by injecting horses with bacteria-free filtrates of young cultures of streptococci, and were therefore as purely antitoxic as could be prepared. (2) The serum of rabbits highly immunised against washed (toxin free) streptococci, though rich in agglutinins, had no protective value. (3) Human convalescent serum had a small but definite protective value. (4) The protective power of antitoxic sera against culture was lost if the serum had previously been treated with an adequate amount of toxin. (5) Scarlet fever toxin, when given intravenously in sufficiently large quantities, killed 80 to 100 per cent. of rabbits. Scarlet fever antitoxin protected rabbits from death due to toxin, while normal horse serum and concentrated diphtheria antitoxin were without protective value. These observations possibly provide a means of titrating scarlet fever toxin and antitoxin without the use of culture.

These results, further details of which will be published at a later date, in conjunction with the agreement obtained with human tests, suggest, if they do not prove, that the test is a measure of " antitoxin " per se.

It may be added that though scarlet fever antitoxin protects rabbits against hæmolytic streptococci from cases of scarlet fever, it also protects against the septicæmia due to certain other hæmolytic strains which have no obvious connexion with scarlet fever. It is not clear at this stage, therefore, to what extent the test can be used conversely to identify the scarlet fever streptococcus. Before this question can be answered it would be necessary to have much more information about the immunological relationships between streptococci of the hæmolytic group and their toxins than is yet forthcoming.

Summary.

(1) Rabbits can be killed with considerable regularity by intravenous injections of broth cultures of the *Streptococcus scarlatinæ*.

(2) A test is described by means of which scarlet antitoxic sera can be compared among themselves and distinguished from other sera. Scarlet fever antitoxin protects rabbits against the more acute phase of the septicæmia due to the streptococcus. Later, many of the protected animals develop arthritis and other manifestations of subacute infection.

(3) The test is a quantitative one; "good" sera can be distinguished from "poor."

(4) In the case of three pairs of antitoxic sera differences were detected by the rabbit test corresponding to differences on human test.

(5) The method, if confirmed in further work, may provide a means of titrating scarlet fever antitoxic serum in the laboratory.

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